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Disposition of Methylglyoxal bis(Guanylhydrazone) (MGBG, NSC-32946) in Man

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Summary. *A high-pressure liquid chromatographic method was utilized to determine the concentration of the antileukemic agent methylgloxal bis(guanylhydrazone) (MGBG, NSC-32946) in tissue samples obtained at autopsy from patients who received MGBG. In a patient with cholangiocarcinoma who received one course of MGBG (500 mg/m^2), the highest drug concentration was found in normal liver tissue. However, the drug concentration in intrahepatic tumor tissue was only 10% of that found in uninvolved liver. In a patient with acute myelogenous leukemia (AML) who received 12 courses of MGBG therapy, highly infiltrated lymph node tissue was found to contain the highest concentration of MGBG. High concentrations of the drug were also found in liver, spleen, kidney, adrenal, and thyroid. The drug penetrated well into normal brain tissue. After repeated administration, high drug concentrations were found in cerebral and cerebellar gray matter. These studies suggest that there is no selective uptake of MGBG into solid tumors early after drug administration and provide a pharmacologic rationale for testing this agent against endocrine and intracerebral tumors in man.*

Introduction

Methylglyoxal bis(guanylhydrazone) (MGBG, NSC-32946) is an antileukemic agent first discovered by Freedlander and French [3]. Early phase I and phase II trials showed that MGBG had severe toxicity when administered at frequent intermittent doses [4, 6, 9]. However, Knight et al. [5] have recently shown that the toxicity of MGBG may be dramatically reduced by utilizing a weekly intravenous dose schedule. As a result, MGBG is currently being re-evaluated in clinical trials against both hematological and solid tumors.

Studies with [^{14}C]MGBG in man [11] have shown that this agent is cleared rapidly from the plasma but is only slowly excreted in the urine as unchanged drug. In view of early clinical studies which showed MGBG toxicity was cumulative [4], we feel that MGBG may be sequestered in the body at unspecified sites.

Accordingly, we have utilized a high-pressure liquid chromatographic (HPLC) method to quantify MGBG in autopsy tissue samples obtained from two patients who received MGBG to determine the tissue disposition of this agent in man.

Materials and Methods

Patients. Patient 1 was a 64-year-old female with a cholangiocarcinoma. She had abnormal hepatic function with marked elevation of her bilirubin and alkaline phosphatase and mild elevation of her lactate dehydrogenase and serum glutamic-oxaloacetic transaminase. In addition, she had a serum creatinine of 3.0 mg/dl and a blood urea nitrogen of 81 mg/dl. She expired 1 h after receiving one course of MGBG (500 mg/m^2) as a 1-h iv infusion. An autopsy was performed 24 h later, which showed cholangiocarcinoma involving 80% of the liver with marked secondary cholestasis. In addition, she had chronic and acute pancreatitis, chronic passive congestion of the spleen, and moderate myocardial hypertrophy. Various tissue samples were excised at the time of autopsy and frozen until analysis.

Patient 2 was a 17-year-old female with AML. She had abnormal liver function with marked elevations of alkaline phosphatase, lactic dehydrogenase, and serum glutamic-oxalacetic transaminase. However, values for bilirubin, serum creatinine, and blood urea nitrogen were all within normal values. The patient received 12 courses of MGBG (400 mg/m^2 each course) therapy at the rate of two courses per week for 6 weeks. The patient expired

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The abbreviations used in this paper are: MGBG, methylglyoxal bis(guanylhydrazone); TCA, trichloroacetic acid; PCA, perchloric acid; KOH, potassium hydroxide; and HPLC, high-pressure liquid chromatography

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24 h after the 12th course. An autopsy performed 24 h later showed extensive leukemic infiltrates in bone marrow, lymph nodes, spleen, liver, kidneys, lungs, epicardium pancreas, and cranial dura. Tissue samples were excised at the time of autopsy and frozen until analysis.

Materials. MGBG was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, MD. Reagents and chemicals were all of HPLC grade or higher, and were purchased from regular commercial suppliers. Methanol distilled in glass was purchased from Burdick and Jackson, Muskegon, MI, USA. Bio-Rex 70 cation exchange resin (200–400 mesh) was purchased from Bio-Rad Labs., Richmond, CA, USA.

Sample Preparation. The quantitation of MGBG in brain tissue has been described previously [12]. Briefly, 0.5-g samples were weighed into 1.5-ml plastic centrifuge tubes. A 0.5-ml aliquot of cold 5% TCA was added and the tissues were disrupted by sonication (Fisher Sonic Dismembrator) and centrifuged at 8,000 *g* for 20 min. The supernatant was decanted into plastic centrifuge tubes and excess TCA was removed by washing twice with cold diethyl ether (Et_2O). After removal of the ether, the samples were adjusted to neutrality with 20 µl 10% Na_2CO_3 and assayed in duplicate for MGBG by HPLC as described below.

Tissue samples (0.5 g) from other organs were weighed into 10-ml plastic centrifuge tubes and a 2.0-ml aliquot of 0.9% NaCl was added. The samples were homogenized with a Polytron tissue homogenizer (Brinkman Instruments) and a 200-µl aliquot of 10 *N* PCA was added. The samples were mixed thoroughly, allowed to stand in ice for 15 min, and centrifuged at 8,000 *g* for 20 min. The supernatants were decanted into plastic centrifuge tubes containing 190 µl cold 10 *N* KOH and were again mixed rapidly and centrifuged at 2,000 *g* for 20 min. The supernatants were then decanted into plastic centrifuge tubes and adjusted to pH 7–8 with 1.0 *N* KOH. These samples were applied to washed Bio-Rex 70 cation exchange resin columns constructed from 1 ml Eppendorf pipet tips (from Curtin Matheson Scientific Inc., Houston, TX, USA). A small plug of glass wool placed in the end of the pipet tip prevented loss of the resin. The resin bed measured approximately 3 cm × 0.5 cm. After application of the sample to the top of the resin bed, the columns were washed with 3 ml distilled H_2O and 3 ml 0.5 *M* sodium acetate buffer (pH 4.0). MGBG was eluted from the resin with 1.9 ml 1 *N* HCL. The eluate was collected in glass test tubes and adjusted to neutrality with 10 *N* KOH. The samples were then analyzed by HPLC as described below. Recovery rates and standard curves for MGBG in tissue samples were constructed by obtaining samples from autopsy of patients who had not received MGBG and by adding various amounts of MGBG to tissue samples prior to homogenization.

Drug Analysis. Analyses of MGBG in tissues were performed with a Waters high-pressure liquid chromatograph with Waters accessories consisting of an Intelligent Sample Processor (WISP model 710A), a model 6000A pump, a model 450 variable wavelength UV detector, and a data module. An analytical reverse-phase µ Bondapack C_{18} column (Waters, 4 mm × 30 cm) was used for separation. The mobile phase was 0.03 *M* sodium acetate buffer, pH 4.3, containing 5% methanol. The flow rate was 2 ml/min and the column eluate was monitored at 283 nm [10].

Results

The HPLC and quantitation of MGBG in biological fluids and brain tissue have been described previously [10, 12]. The present method for the extraction of MGBG from tissue other than brain tissue is an adaptation of the method developed by Marsh et al. [7]. In the present study, various tissue samples obtained at autopsy from patients who had not received MGBG were used for chromatographic blanks and to construct standard curves for MGBG. As shown in Fig. 1A, the MGBG peak was eluted from the column 9.2 min after injection. A representative tissue blank of skeletal muscle (Fig. 1B) shows the absence of materials that might interfere with MGBG determination. A chromatogram of striated muscle from patient 2 is shown in Fig. 1C.

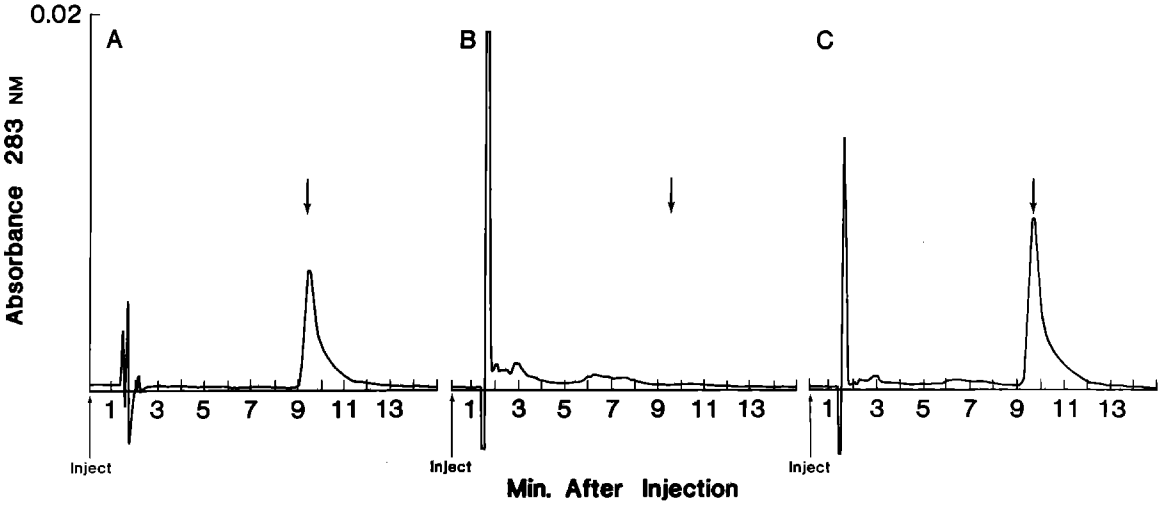

Fig. 1A–C. High-pressure liquid chromatography of MGBG. **A** MGBG standard; **B** Representative chromatogram of tissue blank (striated muscle); **C** MGBG in striated muscle from patient 2. *Arrows* (↓) indicate the position of the authentic MGBG peak

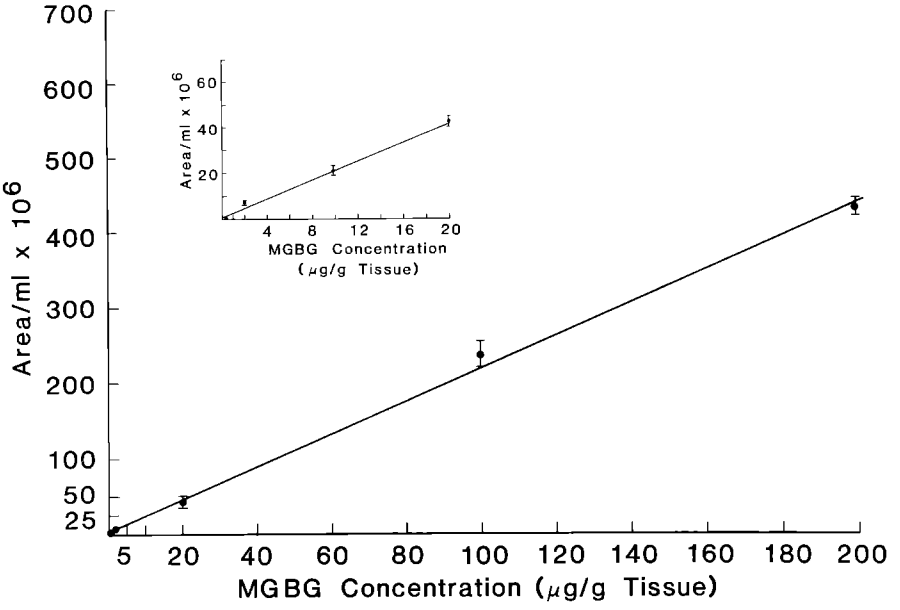

Fig. 2. Representative standard curve for MGBG in tissue is standard curve for MGBG in striated muscle from 0.25–200 μg MGBG/g tissue. Values shown are the means ± SEM for duplicates of three separate determinations

In all tissues studied, prior cation exchange chromatography of the tissue homogenate completely eliminated interference by endogenous substances. Furthermore, standard curves for MGBG in various tissue were linear over the range of 0.25–200 μg/g tissue and were highly reproducible (r^2 between 0.95 and 1.0). Recovery rates for the drug were between 75% and 80%. A representative standard curve for MGBG in skeletal muscle tissue is shown in Fig. 2.

The disposition of MGBG in the tissue of a patient (patient 1) who expired 1 h after receiving one course of therapy (500 mg/m^2, 750 mg total) is shown in Fig. 3. Liver tissue contained by far, the most MGBG. However, the MGBG content in adjacent intrahepatic tumor tissue was only 10% of that in uninvolved liver. Kidney, hepatic tumor, pancreas, lung, and heart all contained similar concentrations of MGBG. Adipose tissue contained the lowest amount of drug. The disposition of MGBG in a patient who received 12 courses of therapy (400 mg/m^2, 600 mg total for each course) was significantly different, as shown in Fig. 4. Although liver contained large amounts of MGBG, drug concentrations in spleen and kidney were much higher than those in patient 1. Surprisingly, lymph node tissue in patient 2 contained the highest concentration of MGBG. Furthermore, adrenal and thyroid tissue also contained substantial amounts of the drug. As in patient 1, the adipose tissue of patient 2 contained the lowest drug concentration.

The penetration of MGBG into various areas of normal brain in patient 1 is shown in Fig. 5. The concentration of MGBG in brain tissue was similar in

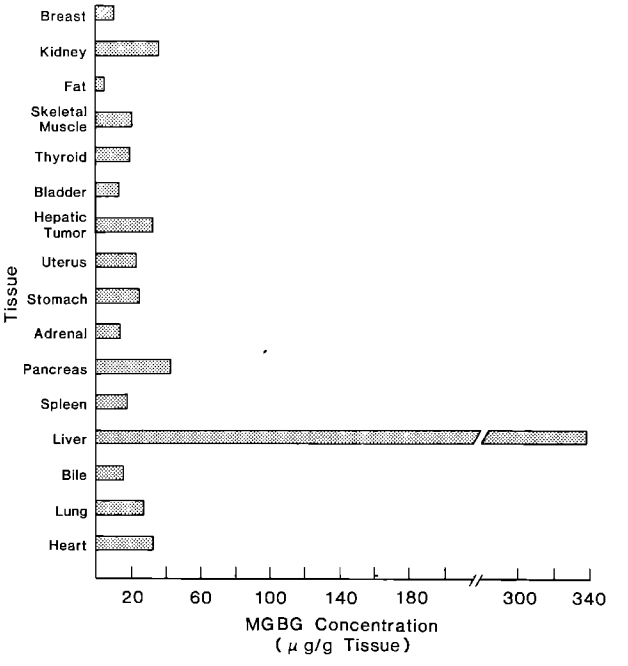

Fig. 3. Tissue distribution of MGBG in patient 1. This patient received one course of MGBG (500 mg/m^2) and expired 1 h after the end of the infusion. Values shown are the means of duplicate determinations

all areas studied (11–14 μg/g tissue) except for cerebral white matter, which contained only 6 μg/g tissue. The penetration of MGBG into the normal brain tissue of patient 2 after 12 courses of therapy was significantly different (Fig. 6). High concentrations of MGBG were found in cerebral and cerebellar gray matter (71.6 and 75 μg/g tissue, respectively).

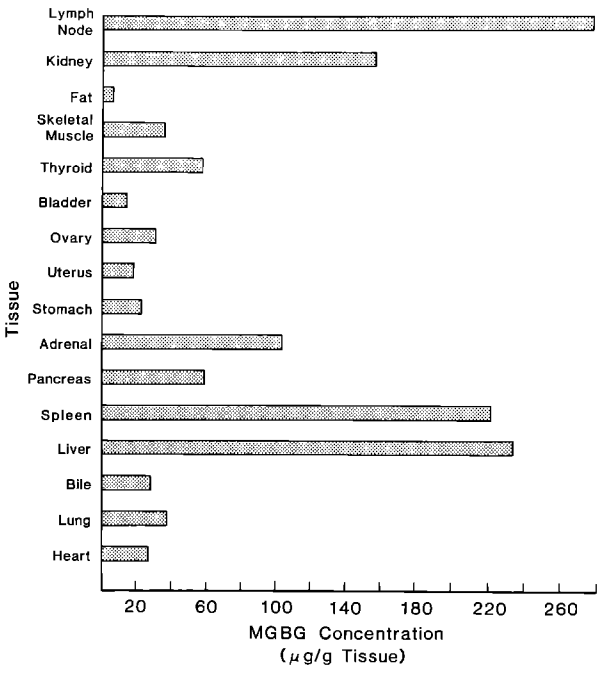

Fig. 4. Tissue distribution of MGBG in patient 2. This patient received 12 courses of MGBG therapy (400 mg/m^2) and expired 24 h after the last course. Values shown are the means of duplicate determinations

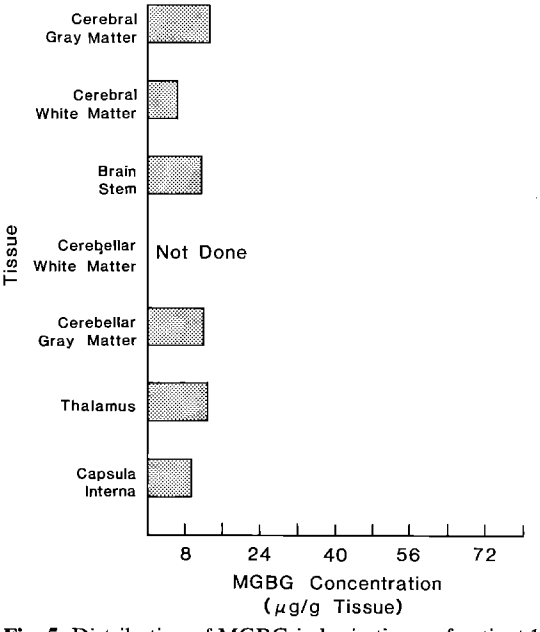

Fig. 5. Distribution of MGBG in brain tissue of patient 1. Values shown are the means of duplicate determinations

Lower drug concentrations were found in the corresponding cerebral and cerebellar white matter (24.6 and 28 μg/g tissue). The ratio of drug in gray matter compared with white matter appeared to be similar: 2.9 for cerebral matter, 2.7 for cerebellar matter.

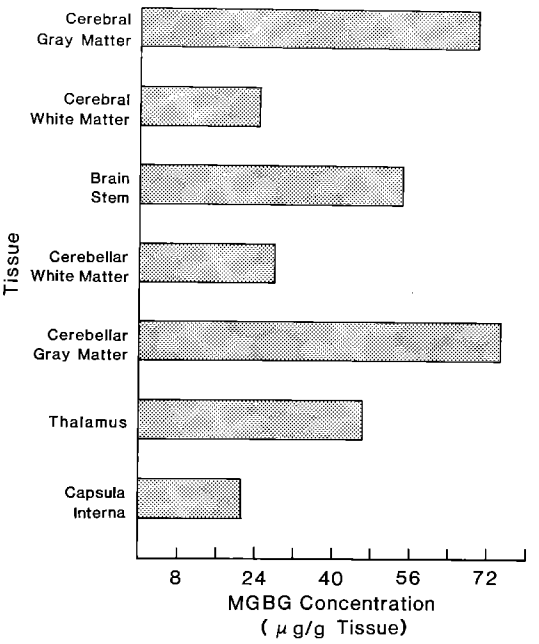

Fig. 6. Distribution of MGBG in brain tissue of patient 2. Values shown are the means of duplicate determinations

Discussion

The tissue distribution of MGBG we observed must be interpreted cautiously, since autopsies were performed 24 h after death, hepatic functions were abnormal in both patients, liver was extensively replaced by tumor in patient 1, and many organs in patient 2 were extensively infiltrated by leukemic cells. On the other hand, the clinical status of these patients was not markedly dissimilar from the status of many patients with advanced neoplastic disease.

Both patients had high concentrations of MGBG in their liver, although drug concentration in bile samples was very low. The reasons for this high hepatic drug concentration are unclear since MGBG is not metabolized to a significant extent by the liver [8]. High drug levels in the liver of patient 2 may be due, in part, to drug uptake by intrahepatic leukemic cells. High concentrations of MGBG were also found in other organs infiltrated by leukemic cells, such as kidney, spleen, lymph node, and pancreas. Previous studies have shown that MGBG is transported into leukemic cells by a carrier-mediated system [2].

We have previously demonstrated that MGBG may achieve concentrations in intracranial tumors that far exceed the concurrent concentrations in plasma [10]. It was therefore surprising that apparently viable intrahepatic tumor in patient 1 contained far less drug than did adjacent uninvolved liver. This

may be explained, in part, by differences in blood flow, since liver receives blood via both hepatic artery and portal vein whereas intrahepatic tumor is supplied by the hepatic artery alone [1].

Patient 2 had substantially higher concentrations of MGBG in both thyroid and adrenal glands than did patient 1. These organs were not infiltrated by leukemic cells in patient 2, and this may imply that endocrine tissues accumulate MGBG with repeated administration.

Patient 2 also had a considerably higher concentration of MGBG in brain tissue than did patient 1. It is unlikely that leukemic infiltration can explain this difference, since only the dura mater was infiltrated. We have previously demonstrated that MGBG achieves high concentrations in intracerebral tumors and low but detectable concentrations in the cerebrospinal fluid of at least some patients after intravenous administration [12]. Since MGBG is highly ionized at physiological pH and has low lipid solubility, it is not clear by what mechanism(s) MGBG penetrates into the central nervous system.

In both patients, less MGBG was found in white matter than in gray matter. White matter has a higher lipid content than does gray matter, and it is unclear whether the difference in lipid content may account for the observed differences in MGBG concentration.

In summary, MGBG appears to localize primarily in liver tissue after drug administration. In addition, in keeping with its known activity in acute leukemia, the MGBG content of organs infiltrated with leukemic cells was high, suggesting that the drug may be concentrated in these cells. Furthermore, MGBG penetrated well into normal brain tissue. The highest concentrations of MGBG were in cerebral and cerebellar gray matter. MGBG appears to gradually accumulate in brain with repeated administration. Finally, in a patient who had received multiple courses of MGBG, moderately high drug concentrations were found in thyroid and adrenal tissue. Therefore, these studies suggest that there may be a pharmacologic rationale for testing this drug against both endocrine and intracerebral tumors in man.

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